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Review of: Overexpression of the colony-stimulating factor (CSF-1) and/or its receptor c-fms in mammary glands of transgenic mice results in hyperplasia and tumor formation

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Abstract of the original article
A number of recent studies have suggested that the colony-stimulating factor (CSF-1) and its receptor c-fms may be involved in the development of mammary glands during lactation and breast cancer. To study the role of CSF-1 or its receptor in initiation of mammary tumorigenesis, we have generated two independent lines of transgenic mice that overexpress either CSF-1 or c-fms under the control of the mouse mammary tumor virus promoter. Mammary glands of the virgin CSF-1 transgenic mice show increased ductal branching, hyperplasia, dysplasia, and other preneoplastic changes, which are indicative of increased cellular proliferation. Similar changes were also evident in the mammary glands of the c-fms transgenic mice. These changes became more prominent with age and resulted in mammary tumor formation. Moreover, secondary events like dimethylbenz(a)anthracene treatment accelerated mammary tumor formation in these mice. Although the expression of estrogen receptor alpha was not significantly changed in either of the transgenic mouse strains, progesterone receptor levels was higher in both transgenic lines as compared with the nontransgenic littermates. Expression of G1 cyclins was prominently increased in the mammary glands of both the CSF-1 and c-fms transgenic lines, suggesting increased cell cycle progression in these strains. In addition, the proliferation marker proliferating cell nuclear antigen (PCNA) and the mitogen-responsive transcription factor c-jun were also increased as compared with the nontransgenic controls. These findings, along with the histological data, support the hypothesis that CSF-1 and its receptor are involved in the etiology of breast cancer.

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Review

In cancers of the reproductive system (breast, endometrium, ovarian, prostate), the macrophage growth factor, colony stimulating factor-1 (CSF-1 or macrophage (M)-CSF), is often over-expressed and this is correlated with poor prognosis [1–5]. CSF-1,

through binding to its single high affinity transmembrane tyrosine kinase receptor (CSF-1R), regulates the survival, proliferation and differentiation of macrophages as well as being chemotactic to these cells [6,7]. The CSF-1R is the product of the *c-fms* proto-oncogene and, in normal mice and humans with the exception of trophoblastic cell expression during pregnancy, its expression is limited to cells of the mononuclear phagocytic lineage [8]. However, in many of these reproductive system cancers, CSF-1R is also expressed in the epithelial cells of tumours concurrently with CSF-1 [1,3]. For example, co-expression of CSF-1 and its receptor is found in ≈50% of late stage breast and ≈70% endometrial cancers [3,4]. Furthermore, in breast cancer, the only case where it has been studied, the infiltration of CSF-1R-expressing macrophages into the tumour is also correlated with poor prognosis [3]. These data suggests that CSF-1 may affect tumour progression by either acting directly on the tumour cells (autocrine) or indirectly (paracrine) through tumour associated macrophages (TAMs). The latter possibility led to the hypothesis that macrophages are recruited to the tumour not to reject it, as was the prevailing view, but to enhance its malignant potential through the trophic and re-modelling capacity of these cells [9]. Indeed, and consistent with this idea, there is now very strong clinical evidence, especially in breast cancers, that the accumulation of TAMs is associated with poor prognosis [10].

The study of Kirma *et al*. [11] sought to investigate the oncogenic potential of CSF-1 and its receptor in the mammary gland *in vivo* by expressing them in an epithelially restricted pattern using the mouse mammary tumour virus (MMTV) promoter in transgenic mice. The effects of both ligand and receptor transgenes were similar in that they caused excessive ductal branching and epithelial hyperplasia at 6–8 months of age and palpable and/or microscopic tumours in 50% of the mice after one year of age. The tumours were typed as adeno- or papillary-carcinomas. Thus, both CSF-1 and CSF-1R are oncogenes in the mammary gland, although weak ones.

The long latency and lack of full penetrance in these transgenic mice suggests that in order for the tumours to develop the accumulation of other oncogenic mutations in the mammary epithelium is needed. Kirma *et al.* [11] therefore, treated the mice with a sub-maximal dose of the chemical carcinogen, DMBA. This increased the incidence and shortened the latency of tumorigenesis in both sets of transgenic mice. Thus, CSF-1 and its receptor are co-operating oncogenes as assessed by this assay.

The over-expression data reported by Kirma *et al*. [11] is essentially the mirror image of previous studies that analysed the effects on mammary development and tumorigenesis in the absence of CSF-1 [12,13] caused by a homozygous null mutation in the CSF-1 gene (*Csf-1*^{op}). In these studies, the mice lacking CSF-1 when compared to wild type mice had a reduction in branching morphogenesis in their mammary gland during puberty that resulted in an atrophic poorly branched structure. Similarly, during pregnancy secondary ductal branching was reduced even through lobuloalveolar development was unaffected [14]. Re-expression of CSF-1 in the mammary epithelium using the MMTV promoter corrected the branching morphogenesis defect while not affecting other phenotypes in the mice showing the organ autonomous nature of the CSF-1 effect [15]. In a cancer context, removal of CSF-1 from a mouse model of breast cancer caused by the mammary epithelial expression of the polyoma middle T-oncoprotein did not affect tumour incidence, but slowed the rate of tumour progression and dramatically decreased metastasis to the lung. Re-introduction of CSF-1 to the mammary epithelium reversed these effects and, in wild type mice accelerated tumour progression and increased the rate of metastasis [12]. In these CSF-1 over-expressing mice, however, effects on tumour formation in normal mammary epithelium were not observed probably because the mice were not followed for long enough [15].

The absence of CSF-1 in these mice resulted in a dramatic reduction of macrophages, both surrounding the developing terminal end buds of the mammary ducts and in the malignant tumours [12,13]. Conversely re-introduction of CSF-1 to the null mutant mammary gland restored the macrophage populations [15]. Similarly, over-expression of CSF-1 in the Kirma *et al*. study [11] resulted in abundant macrophage infiltration to the mammary hyperplasias and tumours. These studies confirm *in vitro* data that CSF-1 is a powerful chemoattractant for macrophages [7] and suggest that this is the cause of the leukocytic infiltration in human tumours where CSF-1 is over-expressed. In the studies described above, using the Polyoma Middle T model of mammary carcinoma, CSF-1R was not found in the mammary epithelium, but was restricted to the infiltrating macrophage population [12]. These data therefore, make a powerful case for TAMs playing an important role in tumorigenesis. The mechanisms for this effect have recently been discussed extensively [16] and given the limitation of space will not be considered in detail here. However, these effects probably involved multiple functions of macrophages that include their angiogenic capacity, their production of matrix re-modelling proteases and growth factors that promote tumour cell proliferation and motility [16,17].

Intriguingly, the results of Kirma *et al*. [11] show that macrophage recruitment to the mammary epithelium on its own is sufficient to cause hyperplasia and

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tumour formation. Given the long latency, this may be through a promotion effect on randomly occurring oncogenic mutations in the mammary epithelium. However, another possibility is that excessive and continuous macrophage recruitment sets up a pseudo-inflammatory response that creates a mutagenic environment through the production of free oxygen and nitrogen radicals that initiates tumour formation through causing oncogenic mutations in the epithelial cells. If this were the case, it couples into a growing body of evidence that links cancer initiation to continuous inflammatory responses caused by chronic infections or irritants [16,18].

The studies of Kirma *et al*. [11], along with the clinical data also suggests an autocrine role for CSF-1 acting through its receptor expressed in the epithelial tumour cells. Studies on cell lines in monolayer culture or mammary epithelial cells in 3-D cultures have indicated that this autocrine loop can promote epithelial cell invasiveness into the surrounding matrix [19,20]. Kirma *et al.* also showed that cellular proliferation was enhanced in the mammary epithelium probably through up-regulation of cyclin D1, a known CSF-1R target gene [21]. Thus the expression of CSF-1R in the mammary epithelium promotes tumorigenesis by the combined action of causing inappropriate cell division and enhancing migration of these cells away from their normal acinar location.

Altogether, the studies in mice and humans create a compelling case for CSF-1 and the CSF-1R having a causal role in at least mammary tumour progression, and probably other cancers. This is either through direct roles in the epithelial cells or through the intermediary of TAMs. In either case, it opens up the possibility of novel therapeutics directed against CSF-1R signalling. Recent pre-clinical studies in mice support this possibility. Treatment with anti-sense or si-RNAs directed against mouse CSF-1 or CSF-1R of transplantable human tumours grafted into nude mice reduced the rate of tumour growth and metastasis [22,23]. Evidence was presented that these effects were through the suppression of TAMs since these tumours did not express CSF-1R and the inhibitory oligonucleotides were targeted against mouse and not human CSF-1. This depletion of TAMs reduced angiogenesis through VEGF-associated mechanisms. These results, together with those showing inhibited tumour progression and metastasis following removal of macrophages by genetic means described above [12], are highly encouraging and indicate the need to develop more refined anti-CSF-1R or anti-macrophage (migration inhibitors?) that can become part of the arsenal of therapies against solid tumours. The link of TAMs to inflammation and its role in carcinogenesis also suggests that creating an anti-inflammatory environment would be beneficial. For example, the observed reduction in cancer risk from Cox-2 inhibitors [24] could well be due in part to effects on macrophages, which are potent producers of prostaglandins and are central players in the inflammatory response.

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